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(*Z*)6,(*E*)8-HENEICOSADIEN-11-ONE: SYNERGISTIC SEX PHEROMONE COMPONENT OF DOUGLAS-FIR TUSSOCK MOTH, *Orgyia pseudotsugata* (McDUNNOUGH) (LEPIDOPTERA: LYMANTRIIDAE)

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Abstract—Three candidate sex pheromone components, (*Z*)6,(*Z*)9-, (*Z*)6,(*E*)8-, and (*Z*)6,(*E*)9-heneicosadien-11-one (Z6Z9, Z6E8, and Z6E9) were identified in pheromone gland extracts of female Douglas-fir tussock moths (DFTM), *Orgyia pseudotsugata* (McDunnough). Their occurrence in subnanogram quantities in extracts and structural conversion during analytical procedures and bioassays complicated chemical identifications. Complete identification required comparative analyses of stereoselectively synthesized and female-produced dienones by coupled gas chromatographic-electroantennographic detection (GC-EAD), high-performance liquid chromatography (HPLC) and coupled GC-mass spectrometry (MS). Determination of the pheromone component was contingent upon an experimental design that minimized structural rearrangement of dienones before and during the field test. In a 40-min field experiment, acetonitrile solutions of each of the above dienones were carried on Dry Ice to traps and were syringed onto cotton release devices below trap lids. In combination with the previously known sex pheromone component of DFTM, (*Z*)6-heneicosen-11-one (Z6), Z6E8 was the only synergistic dienone and the mixture was highly attractive. Because Z6 by itself

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attracts seven species of tussock moths (two sympatric with DFTM), a blend of Z6 and Z6E8 may impart specificity to DFTM pheromone communication. In commercial lures, this binary blend may facilitate species-specific, sensitive monitoring and efficacious control by mating disruption of this important forest defoliator.

Key Words—Lepidoptera, Lymantriidae, *Orgyia pseudotsugata*, tussock moth, (*Z*)6,(*Z*)9-heneicosadien-11-one, (*Z*)6,(*E*)8-heneicosadien-11-one, (*Z*)6,(*Z*)9-heneicosadien-11-one, sex pheromone, synergism.

INTRODUCTION

The Douglas-fir tussock moth (DFTM), *Orgyia pseudotsugata* (McDunnough), is one of the most important defoliators of Douglas-fir and true firs in Western North America (Wickman, 1978; Mason and Wickman, 1988). For example, when 279,000 ha of forest were defoliated in 1974, the Environmental Protection Agency (Federal Register 39, No. 44, pp. 8377–8381) provided an emergency exemption that allowed application of the banned insecticide DDT to 161,000 ha to contain the outbreak. Following the discovery of the sex pheromone component (*Z*)6-heneicosen-11-one (Z6) (Smith et al., 1975), DFTM infestations have been detected and monitored with traps baited with synthetic pheromone (Daterman, 1978, 1980, 1982; Daterman et al., 1979; Shepherd and Otvos, 1986). However, Z6 unspecifically attracts seven species of tussock moths, including whitemarked tussock moth (WMTM), *O. leucostigma* (J. E. Smith), rusty tussock moth, *O. antiqua* (L.), and western tussock moth, *O. cana* Edwards (Daterman et al., 1976; Grant, 1977). Moreover, Z6 is less attractive than unmated DFTM females, supporting the contention that female DFTM produce an additional sex pheromone component (Daterman et al., 1976). This paper describes the identification and field testing of a highly synergistic DFTM sex pheromone component.

METHODS AND MATERIALS

Laboratory Analyses, Instruments, and General Procedures

DFTM male and female pupae were collected around Kamloops, British Columbia. WMTM pupae were reared in Sault Ste. Marie, Ontario, and sent to Simon Fraser University. Male and female pupae were kept separately in filter paper-lined petri dishes at 24°C and under a 14:10 (L:D) photoperiod. At the onset of the scotophase for DFTM or photophase for WMTM (Grant et al., 1975), pheromone glands of 2-day-old virgin females were removed and extracted for 5 min in hexane.

Aliquots of 1 female equivalent (FE) of gland extract were analyzed by

coupled gas chromatographic–electroantennographic detection (GC–EAD) (Arn et al., 1975), using a Hewlett Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25-mm ID) coated with either DB-210 or DB-5 (J&W Scientific, Folsom, CA 95630. GC–mass spectrometry (MS) of synthetic or antennally active compounds in full-scan or selected ion monitoring (SIM) modes with isobutane for chemical ionization (CI) employed an HP 5985B GC–MS equipped with a DB-210 column.

Fractionation of DFTM pheromone extract by high-performanc liquid chromatography (HPLC) employed a Waters LC 625 high-performance liquid chromatograph equipped with a Waters 486 variable-wavelength UV–visible detector set at 220 nm, an HP 3396 series II integrator, and a Nova-Pak C_{18} (3.9 × 300-mm) column with 1 ml/min of acetonitrile flow. Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on a Bruker AMX-400 spectrometer at 400.13 and 100.62 MHz for ^{1}H and ^{13}C NMR spectra, respectively. ^{1}H chemical shifts are reported as parts per million (ppm; δ) relative to TMS (0.00 ppm). ^{13}C chemical shifts are referenced to $CDCl_3$ (77.0 ppm). Two-dimensional correlated spectroscopy (COSY) was used to identify J-coupled protons, while total correlation spectroscopy (TOCSY) was used to identify spin coupled networks. Combined use of both techniques allowed unambiguous assignment of all resonances, even for the mixed rearranged products. Two-dimensional nuclear Overhauser effect spectroscopy (NOESY) was then employed to assign the *E* or *Z* stereochemistry about the olefin bonds.

Elemental analyses were performed using a Carlo Erba Model 1106 elemental analyzer. Chemicals obtained from commercial sources were used without further purification unless otherwise indicated. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography using silica gel 60 (230- to 400-mesh, E. Merck, Darmstadt) (Still et al., 1978). Thin-layer chromatography (TLC) was conducted on aluminum-backed plates precoated with Merck silica gel 60F-254 as the adsorbent and visualized by treatment with an acidic solution of 1% $Ce(SO_4)_2$ and 1.5% molybdic acid followed by gentle heating.

Syntheses

1,4-Decadiyne (1) (Scheme 1, Figure 1). 1-Heptyne (7.2 g, 75 mmol) was added to freshly prepared ethylmagnesium bromide (83 mmol) in Et_2O (150 ml). The mixture was refluxed 3 hr, then cooled to 0°C and CuI (1.25 g, 6.7 mol) in THF (200 ml) was added. After stirring 2 hr, propargyl tosylate (15.75 g, 75 mmol) in THF (50 ml) was added. After 1.5 hr, the reaction mixture was allowed to warm to room temperature (rt) and was stirred 1 hr. A solution of NH_4Cl (15 g in 200 ml of H_2O) was added and the organic layer separated. The remaining aqueous layer was extracted (3 × 50 ml) with ether. Extracts were

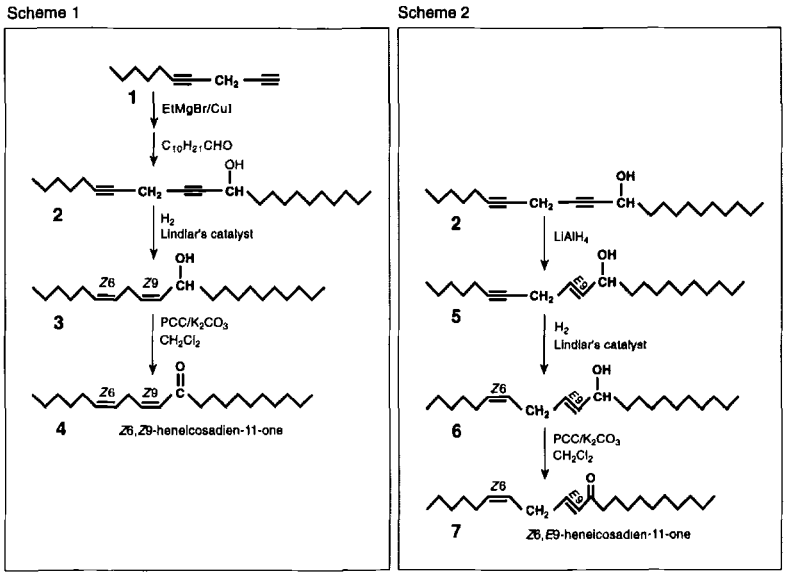

FIG. 1. Routes for the syntheses of (*Z*)6,(*Z*)9-heneicosadien-11-one (**4**; Scheme 1) and (*Z*)6,(*E*)9-heneicosadien-11-one (**7**; Scheme 2).

combined with the organic phase, dried over Na_2SO_4, and distilled *in vacuo* to yield **1** (7 g, 92–96°C/15–18 mm) with a GC purity of 93%. (lit: 75–77°C/12 mm) (Rachlin et al., 1961; Verkruijsse and Hasselaar, 1979). Immediately after distillation the compound began to turn red–yellow and it was used without further purification.

6,9-Heneicosadiyne-11-ol **(2)** *(Scheme 1).* A freshly prepared solution containing 1 equiv of ethylmagnesium bromide was added to **1** (6.13 g, 46 mmol) in ether (150 ml) and the mixture was refluxed 2 hr. After cooling the reaction mixture to −30°C, 0.1 equiv of CuI was added and the mixture stirred 2.5 hr. Following dropwise addition of undecanal (9.41 ml, 46 mmol), the mixture was stirred 1 hr at −20°C, and overnight at rt, and then quenched by the addition of aqueous NH_4Cl. Extraction (3 × 100 ml) with ether:hexane (1:1), separation and washing of organic layers with saturated NaCl, drying, and chromatography (ether/hexane) gave **2** (11.8 g, 85% yield), which was immediately used for the next step. *Anal.* Calcd. for $C_{21}H_{36}O$: C, 82.85; H, 11.92. Found: C, 82.35; H, 11.80.

(Z)6,(Z)9-Heneicosadien-11-ol **(3)** *(Scheme 1).* Alcohol **2** (4.5 g, 14.8 mmol) was hydrogenated using P-2 Ni (or Lindlar catalyst with quinoline), with GC monitoring of the reaction until intermediate monoynes had disappeared.

The resulting dienols were separated by chromatography [SiO_2 (200 g) plus Ag NO_3 (40 g)] using Et_2O/hexanes/toluene (5:45:50) as eluents. Through differential retainment of isomers, pure **3** (2 g, 44% yield) was obtained. *Anal.* Calcd. for $C_{21}H_{40}O$: C, 81.74; H, 13.07. Found: C, 81.90; H, 12.95. ^1H NMR: 5.27–5.49 (m, 4H), 4.46 (dt, 1H), 2.83 (m, 2H), 2.04 (m, 2H), 1.19–1.63 (m, 24H), 0.85 (m, 6H). ^{13}C NMR: 132.94, 130.83, 130.32, 127.15, 67.78, 39.75, 37.54, 31.57, 31.53, 29.61, 29.31, 29.27, 27.26, 26.09, 25.38, 22.66, 22.63, 22.55, 14.04, 13.99.

(Z)6,(Z)9-Heneicosadien-11-one ***(4)*** *(Scheme 1).* Alcohol **3** (100 mg, 3.25×10^{-1} mmol) in CH_2Cl_2 (0.8 ml) at rt was stirred with K_2CO_3 (34 mg, 2.4×10^{-1} mmol) and PCC (105 mg, 4.9×10^{-1} mmol). After 45–50 min of vigorous stirring, hexanes (5 ml) were added and the mixture was flushed through a silica column (20 g) using hexane:ether (99:1) as eluent. The dienone containing fraction, as monitored by GC, was evaporated *in vacuo* at 20–25°C to give **4** (90 mg, 91% yield): *Anal.* Calcd. for $C_{21}H_{38}O$: C, 82.28; H, 12.50. Found: C, 81.92; H, 12.70. ^1H NMR (taken immediately after evaporation): 6.13 (dt, 1H), 5.98 (dt, 1H), 5.44 (m, 1H), 5.36 (m, 1H), 3.38 (dd, 2H), 2.43 (t, 2H), 2.03 (dt, 2H), 1.60 (m, 2H), 1.18–1.38 (m, 20H), 0.85 (m, 6H).

(E)9-Heneicosen-6-yn-11-ol ***(5)*** *(Scheme 2, Figure 1).* Fresh alcohol **2** (3.04 g, 10 mmol) was refluxed 20 hr in ether with lithium aluminum hydride (0.7 g, 20 mmol), and the reaction mixture quenched by slow addition of 2 N NaOH. The precipitate was filtered and washed (3 × 150 ml) with ether. Organic solutions were combined, dried, and evaporated to yield **5** (2.5 g, 82% yield, 97% by GC). ^1H NMR: 5.72–5.79 (M, 1H), 5.60–5.68 (m, 1H), 4.10 (dt, 1H), 2.92 (m, 2H), 2.17 (m, 2H), 1.18–1.55 (m, 24H), 0.87 (m, 6H).

(Z)6,(E)9-Heneicosadien-11-ol ***(6)*** *(Scheme 2).* Alcohol **5** (3.04 g, 10 mmol) was added to a suspension of Lindlar catalyst (0.4 g, 5% Pd on calcium carbonate, poisoned with lead), hexanes (100 ml), and quinoline (1 ml). The presaturated hydrogen mixture was vigorously stirred at rt and hydrogen bubbled through 6 hr, after which the reaction was complete, as monitored by GC over a 10-min period. Ten percent HCl (50 ml) was added with stirring, followed by ether/hexane (1:1) extraction. Combined extracts were washed with water, dried, and evaporated. Purification of crude dienol on a silver column using Et_2O/hexanes/toluene (5:45:50) as eluents yielded **6** (2.4 g, 78%). *Anal.* Calcd. for $C_{21}H_{40}O$: C, 81.74; H, 13.07. Found: C, 81.62; H, 12.93. ^1H NMR: 5.58–5.66 (m, 1H), 5.31–5.51 (m, 3H), 4.04 (dt, 1H), 2.77 (dd, 2H), 2.02 (q, 2H), 1.19–1.57 (m, 24H), 0.81 (m, 6H).

(Z)6,(E)9-Heneicosadien-11-one ***(7)*** *(Scheme 2).* This ketone was obtained (85% yield) from **6** following the same procedure as for conversion of compound **3** to **4** in Scheme 1. ^1H NMR: 6.79 (dt, 1H), 6.10 (td, 1H), 5.54 (m, 1H), 5.37 (m, 1H), 2.95 (t, 2H), 2.51 (dd, 2H), 2.02 (dt, 2H), 1.59 (m, 2H), 1.19–1.48 (m, 20H), 0.88 (m, 6H).

(Z)2-Octenyltriphenylphosphonium Chloride (10) (Scheme 3a, Figure 2). To (Z)2-octenol **(8)** (10 g, 78 mmol) and collidine (10.4 ml, 78 mmol) in DMFA (50 ml) under argon at −30 to −40°C, methanesulfonyl chloride (6 ml, 78 mmol) in DMFA (20 ml) was added dropwise. After stirring 1 hr, the mixture was warmed to 0°C and stirred 1 hr. LiCl (0.1 mol) was added and after 3 hr the mixture was warmed to rt overnight. Water (100 ml) was added and the mixture extracted with hexanes (5 × 50 ml). Combined extracts were washed (2×) with water, dried, and evaporated to yield crude (Z)2-octenyl chloride, which was purified by silica (100 g) column chromatography. Evaporation of the hexane eluent gave the chloride (11.5 g, 95% pure by GC), which was dissolved in acetonitrile (100 ml) to which triphenylphosphine (21 g, 80 mmol) was added. The mixture was refluxed overnight, the solvent evaporated, and the precipitate washed (3 × 100 ml) with hexane/ether (3:1) to yield **10** (26.4 g, 83% yield), m.p. 147–149°C (recrystallized from ethyl acetate). *Anal.* Calcd. for $C_{26}H_{30}ClP$: C, 76.36; H, 7.39. Found: C, 76.44; H, 7.50. ^1H NMR: 7.62–7.88 (m, 15H), 5.70 (m, 1H), 5.37 (m, 1H), 4.69 (m, 2H), 1.63 (m, 2H), 1.10 (m, 2H), 1.03 (m, 4H), 0.74 (t, 3H).

4-Acetoxytetradec-1-ene (4) (Scheme 3b, Figure 2). To freshly prepared vinylmagnesium bromide (30 mmol) in ether (200 ml) was added undecanal (4.8 ml, 22.8 mmol) in ether (100 ml) at 0°C. The mixture was stirred 1 hr and quenched with 2 N aqueous NH_4Cl. The organic layer was separated and the aqueous layer extracted (3 × 50 ml) with ether. Combined extracts and organic fractions were dried over Na_2SO_4 and evaporated to 4-hydroxytetradec-1-ene **(13)** (Matsuda et al., 1989) (4.5 g, 95% by GC). Without further purification, acetic anhydride (3.3 ml, 35 mmol) in pyridine (20 ml) and a catalytical amount of *N,N*-dimethyl-4-aminopyridine (10 mg) were added. The mixture was stirred overnight and evaporated at low pressure. Water (20 ml) was added and the product extracted (4 × 75 ml) with ether. Extracts were combined and dried to yield crude **14** (Chang et al., 1990), which was purified by column chromatography (Et_2O/hexanes, 1:5) (5.22 g, 95% yield from alcohol). *Anal.* Calcd. for $C_{16}H_{30}O_2$: C, 75.54; H, 11.89. Found: C, 75.60; H 12.20.

3-Acetoxytridecenal (15a) (Scheme 3b). 4-Acetoxytetradec-1-ene **(14)** (3 g, 11.8 mmol) was added to $NaIO_4$ (15 g, 71 mmol) and OsO_4 (60 mg) in 100 ml of dioxane/water (9:1). After 8 hr, water (100 ml) was added to the vigorously stirred solution and organic compounds were extracted (4 × 50 ml) with ether. Combined extracts were washed, dried, and evaporated at 35–37°C *in vacuo*. The resulting **15a** became colored when exposed to air, and according to NMR it was contaminated with 5–7% 2-tridecenal **(15b)** (Chang et al., 1990) [^1H NMR −CH=O 9.77 (**t**) and 9.53 (*d*)]. (Attempts to purify **15a** by chromatography led to further elimination of acetic acid and formation of up to 70% of **15b.**)

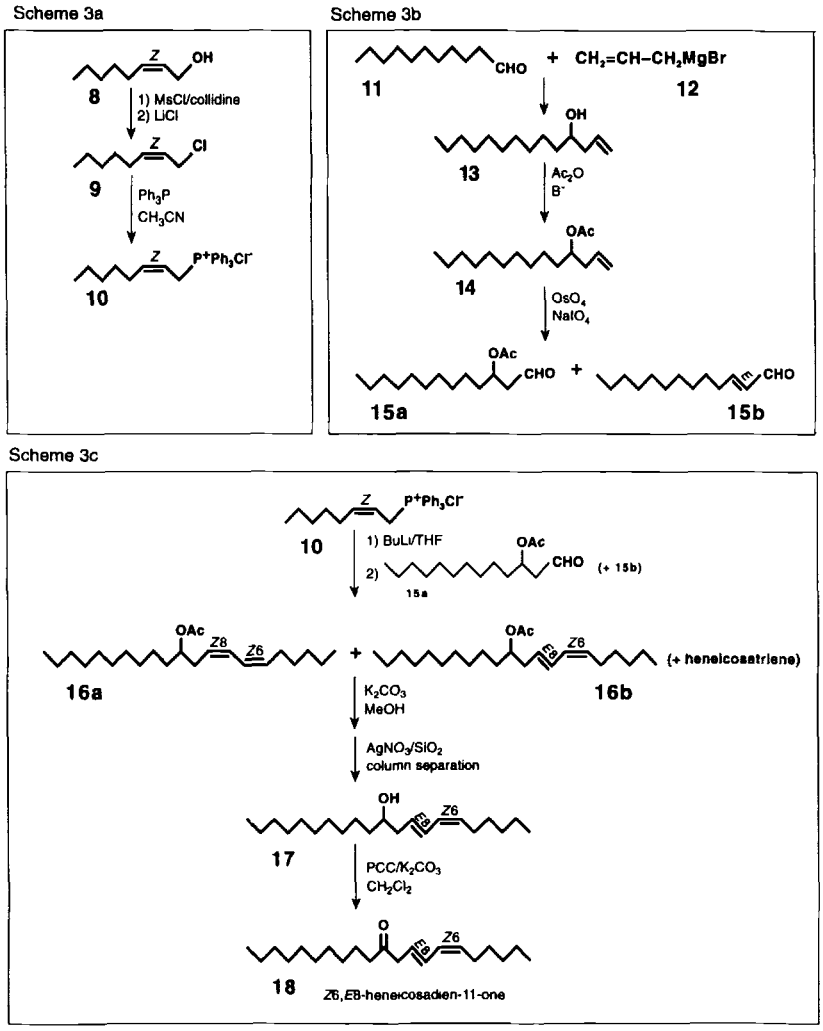

FIG. 2. Routes for the syntheses of (Z)2-octenyl-triphenylphosphonium chloride (**10**; Scheme 3a), 3-acetoxytridecenal (**15a**; Scheme 3b), and (*Z*)6,(*E*)8-heneicosadien-11-one (**18**; Scheme 3c).

(Z)6,(E)8- and (Z)6,(Z)8-Heneicosadien-11-acetates (16a, 16b) (Scheme 3c, Figure 2). All the crude **15a** (Scheme 3b) was dissolved in THF (100 ml) and added slowly to a stirred solution (−78°C) of ylid formed by treating **10** (Scheme 3a) (4.9 g, 12 mmol) in THF (100 ml) with 2.5 M butyllithium in

hexane (5.2 ml) for 2 hr at −70°C. The mixture was warmed to −30 to −20°C and stirred 2 hr, warmed at rt, and quenched with 2 N aqueous NH_4Cl, extracted with ether, dried, and evaporated *in vacuo*. Compounds **16a** and **16b** and a C_{21}-triene formed a 4:6:1 ratio (GC). Diene acetates were purified by chromatography (yield, 2.9 g, 70%). Even with argentation, chromatography separation of *Z,E* and *Z,Z* isomers was difficult. *Anal.* Calcd. for $C_{23}H_{42}O_2$ (mix of isomers): C, 78.79; H, 12.07. Found: C, 78.44; H, 12.12.

(Z)6,(E)8-Heneicosadien-11-ol (17) (Scheme 3c). A mixture of **16a** and **16b** (1 g, 2.85 mmol) was stirred 3 hr with K_2CO_3 (200 mg) in CH_3OH (10 ml). Water (20 ml) was added and alcohols were extracted with ether. Extracts were combined, dried, and evaporated *in vacuo* (0.89 g, 92% yield). The alcohols were separated on an argentation silica column and eluted with a mixture of Et_2O/hexanes/toluene (1.5:48.5:50). Seventy milligrams of earlier-eluting **17** was collected and the remaining mixture, containing both isomers, was used in subsequent column separation. *Anal.* Calcd. for $C_{21}H_{40}O$: C, 81.74; H, 13.07. Found: C, 81.68; H, 13.28. ^{1}H NMR: 6.42 (m, 1H), 5.97 (dd, 1H), 5.64 (m, 1H), 5.37 (dt, 1H), 3.62 (m, 1H), 2.34 (m, 2H), 2.16 (m, 2H), 1.23–1.51 (m, 24H), 0.87 (dt, 6H).

(Z)6,(E)8-Heneicosadien-11-one (18) (Scheme 3c). This ketone (95% yield) was obtained from **17** following the same procedure as for conversion of compound **3** to **4** in Scheme 1. *Anal.* Calcd. for $C_{21}H_{38}$: C, 82.28; H, 12.50. Found: C, 81.82; H, 12.80. ^{1}H NMR: 6.40 (m, 1H), 5.97 (dd, 1H), 5.73 (dt. 1H), 5.41 (dt, 1H), 3.20 (d, 2H), 2.43 (t, 2H), 2.14 (m, 2H), 1.58 (m, 2H), 1.21–1.40 (m, 20H), 0.88 (m, 6H). An improved alternative synthesis for **18** employs $Pd(PPh_3)_4$-catalyzed coupling of (*Z*)-1-iodo-1-heptene with (*E*)-4-(tert-butyldimethylsiloxy)-1-tetradecenylboronic acid (Wimalaratne and Slessor, unpublished).

Field Experiments

Field experiments were conducted in mature Douglas-fir stands west and north of Kamloops, British Columbia. Unitraps (Expts. 1 and 2) (Phero Tech Inc., Delta, British Columba V4G 1E9) or sticky 2-liter Delta milk cartons (Gray et al., 1984) (Expts. 3–6) were suspended from Douglas-fir trees 1.5 m above ground in complete randomized blocks with traps and blocks at >20-m intervals. A small cube of vapona (18.5% dichlorvos; Green Cross, Division of Ciba Geigy Canada Ltd., Mississauga, Ontario L4Z 2Z1) in Unitraps assured retention of captured moths. Traps were baited with cotton dental wicks (Richmond Dental Company, Charlotte, NC 28234) (Expts. 1 and 3–6) cut to 1-cm length or with gray sleeve stoppers (West Company, Lionville, PA 19341) (Expt. 2) baited with candidate pheromone components. Z6 purchased from Phero Tech Inc., was >98% chemically and geometrically pure.

Experiment 1 tested Z6 (1 μg) alone and in combination with Z6E8, Z6Z9, or Z6E9 at 0.01 μg each (Figure 4). During peak flight activity of DFTM males, these HPLC-purified dienones in acetonitrile were carried on Dry Ice to traps and were syringed onto cotton wicks affixed to Unitrap lids. DFTM trap catches were recorded 40 min later. In all subsequent experiments lures were also prepared in the field. The second experiment tested Z6 (100 μg) alone and in combination with Z6E8 (1 μg) (Figure 5). The third experiment tested Z6 (10 μg) alone and in combination with Z6E8 at increasing doses of 0.01, 0.1, and 1 μg (Figure 6). Experiment 4 tested Z6 plus Z6E8 at 10:1 and 10:10 ratios.

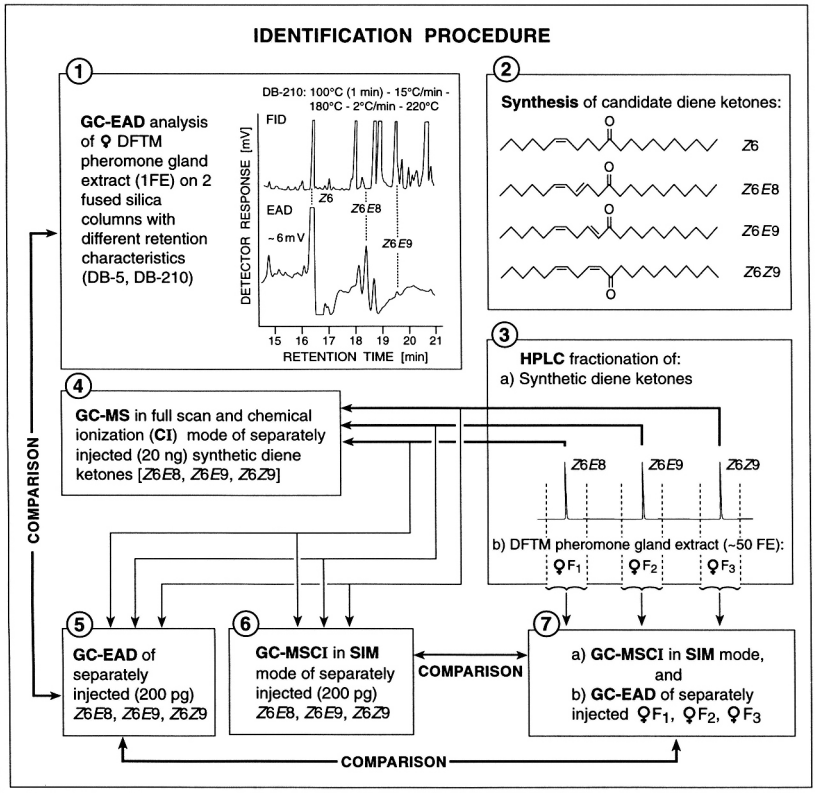

FIG 3. Procedure for the identification of (*Z*)6,(*E*)8-, (*Z*)6,(*E*)9, and (*Z*)6,(*Z*)9-heneicosadien-11-one (Z6E8, Z6E9, Z6Z9) in female Douglas-fir tussock moth (DFTM) pheromone gland extract. FE, female equivalent of pheromone extract; female F1, fraction 1 of female DFTM pheromone gland extract; GC-EAD, gas chromatographic—electroantennographic detection; HPLC, high-performance liquid chromatography; GC-MSCI, gas chromatographic-mass spectrometric analyses in chemical ionization mode; SIM, selected ion monitoring mode.

The fifth experiment tested Z6 (10 μg) in combination with Z6E8 (1 μg) versus either compound alone at 10 μg each (Figure 7). The sixth and final experiment tested Z6 alone at 1, 10, 100, or 500 μg and in binary combination with Z6E8 at 10:1 ratios (Figure 8).

RESULTS AND DISCUSSION

WMTM pheromone analyses by GC-EAD revealed antennal responses to Z6 and several unknown components, one of which gave GC-MS fragmentation ions indicative of a diunsaturated C_{21} ketone. Because a diene ketone, 1,6-heneicosadien-11-one, had previously been identified in DFTM pheromone extracts (Smith et al., 1978), and considering that (*Z*)6,(*Z*)9-diene epoxides are common pheromones in geometrid and arctiid moths (Arn et al., 1992), we synthesized (*Z*)6,(*Z*)9-heneicosadien-11-one (Z6Z9) (Figure 3, box 2). Synthetic Z6Z9 in solution at room temperature and during GC analyses rearranged to (*Z*)6,(*E*)8- and (*Z*)6,(*E*)9-heneicosadien-11-one (Z6E8, Z6E9) and other dienones, as determined by NMR spectroscopy and syntheses of the tentatively

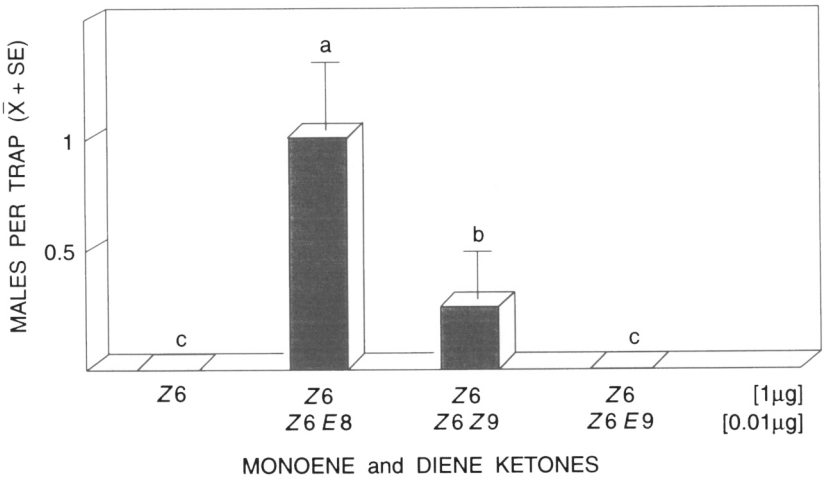

FIG. 4. Capture of male Douglas-fir tussock moths during a 40-min experiment in Unitraps baited with (*Z*)6-heneicosen-11-one (*Z6*) alone and in combination with either (*Z*)6, (*E*)8-, (*Z*)6,(*E*)9-, or (*Z*)6,(*Z*)9-heneicosadien-11-one [Z6E8, Z6E9, Z6Z9]. Kamloops, British Columbia, 7 September 1993, 1830 to 1910; 12 replicates. The experimental time was confined to 40 min to minimize the rearrangement of Z6Z9 to Z6E8 while allowing capture of sufficient numbers of male DFTM to reveal the synergistic pheromone component (most attractive treatment). Bars with the same superscript are not significantly different. Nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) *t* test, $P < 0.05$] (SAS/STAT, 1988).

identified rearrangement products. Identical GC retention times of Z6Z9 and Z6E8 indicated conversion of Z6Z9 to Z6E8, similar but not analogous to deconjugations reported for α,β-unsaturated ketones (Ricard et al., 1986).

GC-EAD analyses of DFTM pheromone extract revealed several compounds consistently eliciting responses by male DFTM antennae, with Z6 being most abundant and (thus) most EAD active (Figure 3, box 1). Comparative GC-EAD on DB-210 and DB-5 columns of pheromone extract and synthetic dienones resulted in retention time matches of two EAD-active, female-produced compounds with synthetic Z6E8 and Z6E9, respectively.

With known HPLC retention times of synthetic Z6E8, Z6E9, and Z6Z9 (which do not rearrange during HPLC), corresponding fractions of DFTM pheromone extract were isolated (Figure 3, box 3). Each synthetic dienone was then analyzed by GC-MS in full scan and CI mode to determine diagnostic ions for SIM (Figure 3, box 4), which provides highly increased sensitivity. GC-MSCI-SIM of synthetic dienones and corresponding DFTM extract fractions (Figure 3, boxes 6 and 7a) resulted in good retention time and ion ratio matches between synthetic and female-produced Z6E8 and Z6E9, respectively [GC-MSCI retention time, *m/z* (relative intensity): synthetic Z6E8—18.6 min, 307 (100), 308

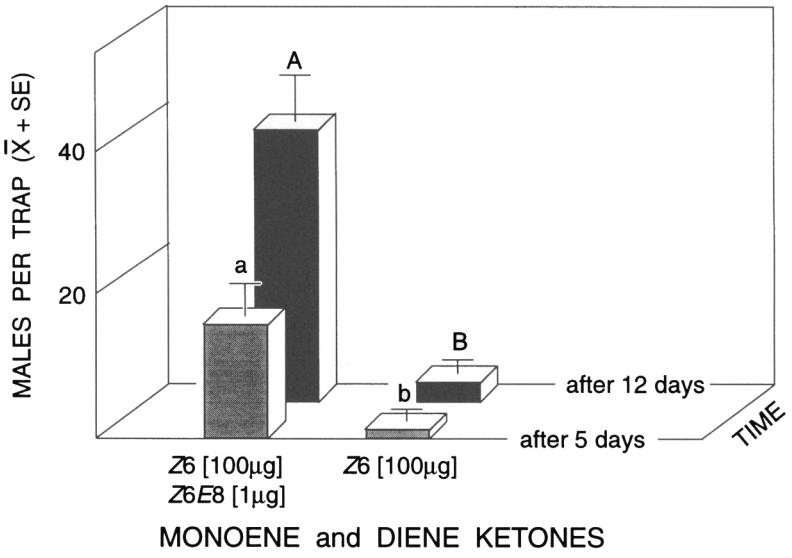

FIG. 5. Capture of Douglas-fir tussock moth males in Unitraps baited with rubber septa impregnated with (*Z*)6-heneicosen-11-one (Z6) alone or in combination with (*Z*)6,(*E*)8-heneicosadien-11-one (Z6E8). Vinsulla, British Columbia, 5–10 September, 1994; 12 replicates. Lures were not changed after 5 days. For each trapping period, treatments were significantly different. Nonparametric Mann-Whitney test, $P < 0.05$.

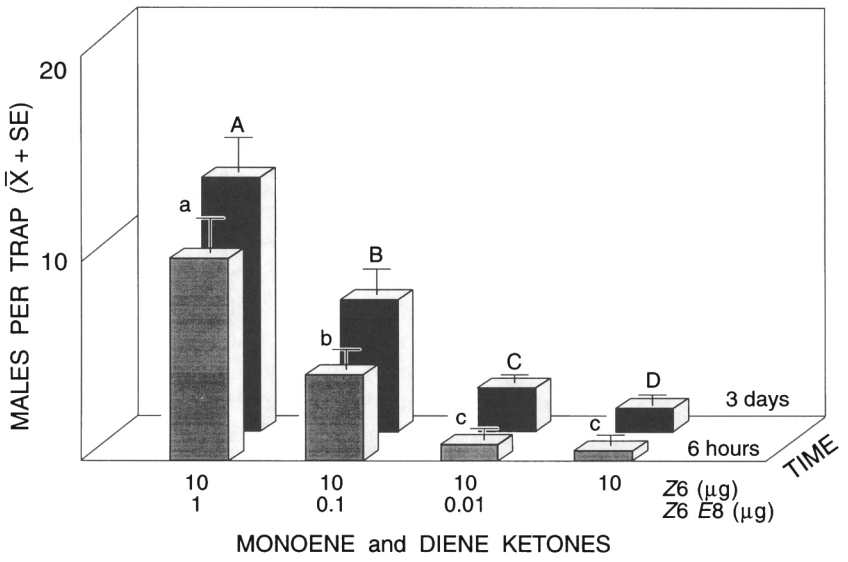

FIG. 6. Captures of Douglas-fir tussock moth males in 2-liter Delta milk carton traps baited with cotton wicks impregnated with (Z)6-heneicosen-11-one (Z6) alone and in combination with (*Z*)6,(*E*)8-heneicosadien-11-one (Z6E8) at increasing proportions. Lures were not changed between trapping periods. Vinsulla, British Columbia, 10–13 September, 1994; 10 replicates. For each trapping period, bars with the same superscript are not significantly different. Nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [(Bonferroni (Dunn) *t* test, $P < 0.05$].

(24); DFTM gland extract—18.6 min, 307 (100), 308 (26); synthetic Z6E9—19.7 min, 307 (100), 308 (23); DFTM gland extract—19.7 min, 307 (100), 308 (33); synthetic Z6Z9[5]—18.6 min, 307 (100), 308 (27); DFTM gland extract—18.6 min, 307 (100), 308 (21)]. During GC–MSCI–SIM, HPLC-fractionated synthetic Z6Z9 and DFTM-dienone in the corresponding HPLC extract fraction (Figure 3, box 3) rearranged to compounds with identical retention and ion ratio characteristics, thereby confirming the presence of Z6Z9 in the DFTM pheromone extract. Moreover, comparative GC–EAD of synthetic dienones (Figure 3, box 5) and corresponding DFTM extract fractions (Figure 3, box 7b) revealed comparable antennal response patterns.

Twenty-four pre-screening field tests, evaluating various trap designs (sticky traps and nonsaturating Unitraps), release devices (rubber septa, cotton, and silica), lure doses (0.01–0.1 μg), and experimental times (40 min to 4 days), led to the critical experiment which determined the synergistic DFTM phero-

[5]Synthetic (*Z*)6,(*Z*)9-heneicosadien-11-one was injected but, due to thermal instability, rearranged on injection, primarily to (*Z*)6,(*E*)8-heneicosadien-11-one.

mone component. Syringing HPLC-purified and Dry Ice-stored dienones onto cotton wicks affixed to lids of presuspended traps, and recording the number of trap-captured DFTM males 40 min later, Z6 alone and in combination with Z6E9 had not attracted any males (Expt. 1, Figure 4). Attraction of males to Z6E8 in combination with Z6 (Expt. 1, Figure 4) indicated that this dienone is a potent sex pheromone component in DFTM. The apparent attractiveness of Z6Z9 in combination with Z6 can be attributed to partial rearrangement of Z6Z9 to Z6E8 during the 40-min test. Attractiveness of Z6 plus Z6E8 dispensed from rubber septa greatly exceeded that of Z6 alone (Expt. 2, Figure 5). Increasing doses of Z6E8 added to Z6 resulted in increasing captures of DFTM males (Expt. 3, Figure 6). At 10:1 or 10:10 ratios, Z6 plus Z6E8 (Expt. 4) attracted on average 16.2 ± 1.7 (SE) and 22.2 ± 2.5 DFTM males (10 replicates; nonparametric Mann–Whitney test, $P > 0.05$). While Z6 or Z6E8 singly were not very attractive, combined they were highly affective in attracting DFTM males (Expt. 5, Figure 7). In the final dose—response experiment, Z6 (10 μg) plus Z6E8 (1 μg) was as attractive as 500 μg of Z6 alone (Expt. 6, Figure 8). Because population densities of sympatric *Orgyia* spp. were very low, congeners of the DFTM were not captured in any of the experiments.

Following the discovery of (Z)6,(Z)9-nonadecadien-3-one in *Peribatodes*

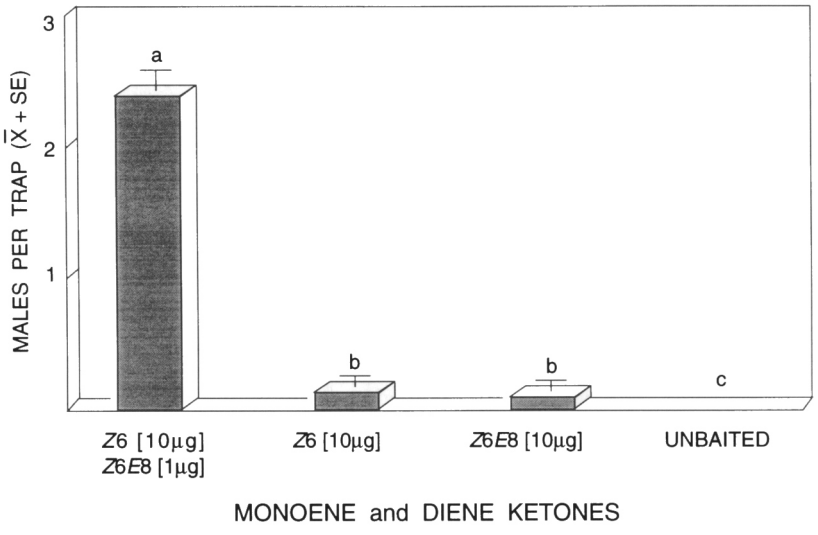

FIG. 7. Captures of Douglas-fir tussock moth males in 2-liter Delta milk carton traps baited with cotton wicks impregnated with (Z)6-heneicosen-11-one (Z6), (Z)6,(E)8-heneicosadien-11-one (Z6E8), or both combined. Vinsulla, British Columbia, 17 September 1994; 1400 to 1845; 10 replicates. Bars with the same superscript are not significantly different. Nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) t test, $P < 0.05$].

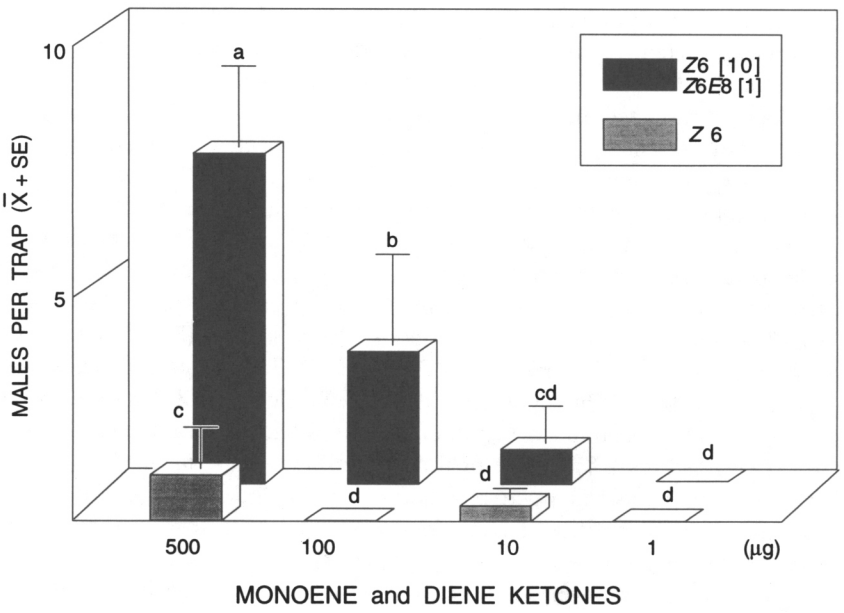

FIG. 8. Captures of Douglas-fir tussock moth males in 2-liter Delta milk carton traps baited with cotton wicks impregnated with increasing doses of (*Z*)6-heneicosen-11-one (Z6) alone or in 10:1 combination with (*Z*)6,(*E*)8-heneicosadien-11-one, Vinsulla, British Columbia, 17–24 September 1994; five replicates. Bars with the same superscript are not significantly different. Nonparamteric analysis of variance by ranks (Friedman's test) followed by comparison of means (Student Newman Keuls test, $P < 0.05$).

rhomboidaria (Buser et al. 1995), Z6E8 is the second dienone sex pheromone component found in the Lepidoptera. Chemical lability and subnanogram quantities of dienones in DFTM pheromone extract required comparative GC–EAD, HPLC, and GC–MSCI–SIM of synthetic and DFTM-produced dienones for identifications. Determination of Z6E8 as the synergistic DFTM pheromone component was contingent upon an experimental design that minimized rearrangement of dienones prior to and during the field test (Expt. 1). Because Z6 is a congeneric sex pheromone component (attractant) in *Orgyia* and *Dasychira* tussock moths (Arn et al., 1992), a blend of Z6 plus Z6E8 (at ratios of 10:0.1, 10:1, or 10:10) may impart specificity to DFTM pheromone communication. It will be of great interest to investigate additional sex pheromone components in other tussock moths. Female WMTM, for example, do not utilize Z6E8 for pheromone communication (Slessor and Grant, unpublished), but their pheromone gland extracts greatly lose attractiveness within 24 hr at room temperature (Slessor and Grant, unpublished), suggesting the presence of a (thermo)labile,

as yet unknown sex pheromone component. Following commercial formulation, the identified two-component DFTM pheromone blend may allow species-specific and highly sensitive monitoring of DFTM populations. It may also greatly enhance the efficacy of pheromone-based control of this important forest defoliator (Sower et al., 1990; Hulme and Gray, 1994).

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